REMARKS

Claims 144, 145, 149, 159, 160, 163, 177, 179, 184, 185, 191, 192 and 195-221 presently appear in this case. No claims have been allowed. The official action of August 10, 2004, has now been carefully studied. Reconsideration and allowance are hereby respectfully urged.

Briefly, the present invention relates to a method for identifying continuous peptides that simulate a discontinuous epitope of a single biological unit, i.e., that interact with a ligand that interacts with a discontinuous epitope of a single biological unit. The single biological unit may be a single protein or a complex of proteins. The DNA that encodes the amino acid sequence of the biological unit is divided into DNA fragments. A library of oligonucleotides is then created, each comprising at least two of such fragments that are randomly ligated. Preferably, the random ligation is such that any oligonucleotide in the library can ligate with any other oligonucleotide in the library. Preferably, this library will contain oligonucleotides of fragment pairs in which each fragment is linked to each other fragment. The oligonucleotides are inserted into an expression system and then expressed. The resultant is then screened for interaction with a ligand that interacts with a discontinuous epitope of the single

biological unit. Those that are identified with such positive interaction are then produced and can serve to simulate the native discontinuous epitope.

Claims 144-156, 159-170, 177, 179 and 183-194 have been rejected under 35 USC 112, first paragraph, as failing to comply with the written description requirement on the grounds that several of the limitations added to several different claims are apparently new matter. The examiner states that the limition "such that any oligonucleotide in the library can ligate with any other oligonucleotide in the library" is effectively new matter. This part of the rejection is respectfully traversed.

The examiner's attention is invited to page 31, lines 19-28, of the present specification. There, it states:

> After the digest, either partial or substantially complete, has been prepared, the resulting fragments are processed so that these fragments have appropriately cut ends, by enzymatic degradation or synthesis for example.

One of ordinary skill in the art would understand this to mean that whatever overhangs remain on the fragments after their generation can be removed by enzymatic degradation or filled in by enzymatic synthesis (Klenow fill-in) in order to produce ligation compatible blunt ends. The only interpretation possible for one of ordinary skill is that blunt ends are

being produced (see also page 18, lines 21-24) and therefore no preference for ligation between any two random fragments is imposed.

Even in the case where a specific restriction enzyme is used, see for example, page 31, lines 1-7, one would expect to get a multiplicity of fragments, each having the same overhang. Since they all have the same overhang, they would be expected to interchange without any preference for one fragment over another. No specific pairing or orientation would be imposed.

Thus, the concept that any oligonucleotide in the library can ligate with any other oligonucleotide in the library is present as it stems from the very nature of the methodology described on page 31. It is not necessary for claim language to be supported *in ipsis verbis*, as long as the concept of the claim language is present in the specification. See *In re Wertheim*, 191 USPQ 90, 96 (CCPA 1976) ("It is not necessary that the application describe the claim limitations exactly ..., but only so clearly that persons of ordinary skill in the art will recognize from the disclosure that appellants invented processes including those limitations.") The language to which the examiner objects merely makes explicit that which had been implicitly contained in the specification as filed. The written description guidelines

state that the written description requirement of 35 USC 112 can be fulfilled by what is explicitly, implicitly or inherently present in the specification. MPEP 2163 I.B. (page 2100-167, rev. 2, May 2004) and II.A.3.(b) (page 2100-175). That each oligonucleotide may bind to any other oligonucleotide in the library was implicitly part of the specification as originally filed. Accordingly, reconsideration and withdrawal of this part of the rejection is respectfully urged.

The examiner also states that the limition "wherein said providing and creating steps are accomplished such that none of the oligonucleotides created thereby encode said original single biological unit."

In order to obviate this part of the rejection, all claims using this language have now been deleted.

The third limitation to which the examiner objects is "a single definable sequence." This part of the rejection is also respectfully traversed.

Page 12 of the present specification states that the preferred genetic material is from an organism which is a virus, a bacterium, a yeast or a parasite. These typically do not have duplications, pseudogenes, or alternate splicing abilities. They will inherently have a single definable sequence for any given strain. At page 13, line 1, the

specification states that preferably the biological unit is a polypeptide. Such would be considered to have a single definable sequence. The term was intended to read on any sequence which does not have to be mutated prior to shuffling, as in Stemmer, or to avoid the intentional use of many different sequences, as in Huse. The concept of not mutating or using a plurality of different genes is present in the specification as a whole and should be permitted here. Reconsideration and withdrawal of this part of the rejection is also respectfully urged.

It should be noted that the present claims were added in the expectation that they would facilitate allowance, even though applicant was certain that neither Huse nor Stemmer started with a "single biological unit" and neither screen "for interaction with a ligand that interacts with a discontinuous epitope of said single biological unit." As the additional limitations have not resulted in the allowance of any claims, applicant has now taken the liberty of reinserting claims without any of these limitations, i.e., new claims 195-221. These claims are therefore not subject to the present 35 USC 112 rejections. The reason why these claims are not anticipated or made obvious by either Huse or Stemmer will be explained again, in the hope that the examiner will reconsider his previous position and avoid the necessity of appeal.

It is Huse's intent to produce a combinatorial library of Fab fragments of the mouse antibody repertoire in *E. coli*. He effectively makes the same library of monoclonal antibodies that is made by conventional hybridoma technology. At page 1277, in the paragraph bridging columns 1 and 2, Huse states that, via PCR amplification of a total mRNA preparation, he is amplifying all of the Fd and κ chain sequences from the splenocytes of a given mouse, irrespective of their clonal origin. Huse then makes two separate libraries, one containing the total mixture of all heavy chains and one containing the total mixture of all light chains. In view of the fact that each chain of either library is derived from a multitude of different B-cells, such chains represent distinct and different biological units, or polypeptide chains of distinct and different biological units. **At no time does Huse produce a plurality of DNA fragments which appear in the DNA sequence encoding a single biological unit.** Huse starts with DNA encoding a multitude of monoclonal antibodies, i.e., the mRNA from a multitude of different B-cells found in the spleen of an immunized mouse. It is a total distortion of the term "single biological unit" to interpret it to read on a multitude of **different** antibodies. A polyclonal mixture of diverse antibodies is no more a single biological unit because each is an "antibody" than is a

mixture of diverse and different enzymes a single biological unit because each is an "enzyme."

As can be seen from Fig. 1 of Huse, a heavy chain randomly selected from the heavy chain library and a light chain randomly selected from the light chain library are constructed to form a dicistronic mRNA from the lac Z promoter. They are not contiguously ligated as is necessary in the present claims. To highlight this additional difference, all of the claims have now been further amended to specify that each of the oligonucleotides in the library comprises "at least two contiguous fragments." Support for this is found at page 32, lines 15-17, which states that the discontinuous epitopes being prepared by the present invention include "the novel **contiguous** presentation of two different amino acid sequences which were previously discontinuous in the original primary amino acid sequence." Contiguity of the fragments is thus an important and disclosed feature of the present invention.

Furthermore, step (e) of claim 195 requires the step of screening the expressed peptides for interaction with a ligand that interacts with a discontinuous epitope of said single biological unit. Huse does not do this. The examiner states that the antigen used in the antibody screen is such a ligand. However, this is incorrect. Huse reconstitutes

antibodies. The antibody chains are linear epitopes, not discontinuous epitopes. The antigens against which the antibodies of Huse are screened do not interact with a discontinuous epitope. This requirement of the claims cannot be simply ignored. The concept of discontinuous epitopes is explained at page 7 of the specification. As the antigens of Huse interact with a linear or continuous epitope of the antibody that makes up the alleged "single biological unit" of Huse, the step (e) of claim 195 is not anticipated or made obvious by Huse.

For all of these reasons, none of the present claims are anticipated or made obvious by the technology of Huse.

With respect to Stemmer, applicants have previously argued that Stemmer does not start with a single biological unit as he must first mutate the starting protein or antibody, in which case, each mutated protein is a separate and distinct biological unit. The examiner has not been convinced by this argument. Applicant's have now studied Stemmer in more depth and have determined that there is another significant difference between the process of Stemmer and the present claims. **Stemmer does not create oligonucleotides from DNA fragments by random ligation, as is required by the present** claims. Stemmer does not ligate at all. To ligate requires that two strands of DNA or RNA be concatenated using a ligase.

Reference is made to the following definitions obtained from the internet. From

http://onlinedictionary.datasegment.com/word/Ligate:

> **Ligate** - *definition from gcide*
> Ligate \Li"gate\ (l[imac]"g[=a]t), v. t. [L. ligatus, p. p. of ligare.]
> 1. To tie with a ligature; to bind around; to bandage.
> [1913 Webster]
>
> 2. (Molecular biology) To concatenate two strands of (nucleic acid, usually DNA), in an end-to-end fashion, using a ligase.
> [PJC]

From

http://www.informatics.jax.org/mgihome/other/glossary.shtml:

> **Ligate**
> In molecular biology, to join two separate DNA or RNA segments to form a single DNA or RNA molecule enzymatically.

Stemmer does not concatenate strands of DNA, nor does he use a ligase, nor does he join DNA segments to form a single DNA molecule enzymatically. Stemmer uses his DNA fragments as templates for PCR strand extension. At col. 4, lines 38-43, Stemmer explains that he uses conditions suitable for PCR amplification to thereby homologously recombine the fragments to form a shuffled pool of recombined polynucleotides. This is further explained at col. 6, lines 1-20. Note that in order to achieve his objectives, the polynucleotides must first be denatured in order to generate

single stranded fragments which are then incubated such that, through homologous annealing, one strand will serve as primer to the other, while the other serves as a template for the first. In this fashion, through parallel PCR (col. 21, lines 31-51), a single biological unit is reconstituted. This is achieved not by "ligation" of DNA fragments, but rather through polymerization and strand elongation via taq polymerase or the like (not ligase, which is used to concatenate or join two DNA strands).

Accordingly, Stemmer does not create a library in which fragments are "randomly ligated" as is required by all of the present claims and therefore none of the present claims are anticipated by or made obvious by Stemmer.

In addition, Stemmer does not screen expressed peptides for interaction with a ligand that interacts with a discontinuous epitope of the single biological unit. This critical feature of the present invention is simply not present in Stemmer. Any screening is done with ligands that recognize linear or continuous epitopes. Thus, claim 195, and the claims dependent therefrom, are patentable over Stemmer for this reason as well.

Furthermore, many of the dependent claims are patentable in their own right as they add features which are not disclosed by or obvious from either Huse or Stemmer. As

to claim 196-198 and 210-212, Huse does not cut the DNA but creates fragments by PCR amplification. Thus, these claims cannot be anticipated or made obvious by Huse.

Neither Huse nor Stemmer disclose or suggest synthesizing the plurality of DNA fragments, and thus claim 199 is not anticipated or made obvious by either reference.

Claims 200 and 213 require the technique of randomly ligating fragments to make larger fragments and then at least partially digesting the ligated fragment to form the library of oligonucleotides. This is certainly not even remotely suggested by either Huse or Stemmer and thus claims 200 and 213 are patentable in their own right and should be indicated to be allowable over all of the references of record.

As to claim 201 and 214, Huse only teaches display on lambda bacteriophage. Expression in bacteria is not anticipated or made obvious by Huse.

Neither Huse nor Stemmer discloses or makes obvious use of a eukaryotic expression system as claimed in claims 205 and 218.

The single biological unit of Huse is an antibody and not a single protein as is claimed in claims 206 and 219. There would be no reason to substitute a single protein for the two-chain antibody of Huse. The word "single" has been

added before "protein" in order to make explicit what had previously been implicit.

The size of the fragments in claims 208 and 221 is nowhere taught or suggested by Huse. The "fragments" of Huse are entire heavy chains and light chains. The features of claims 208 and 221 are thus not anticipated or made obvious by Huse.

Accordingly, all of the claims discussed above should be considered in their own right with respect to each of the primary references and indicated to be free of the particular rejection where appropriate.

Claims 144-146, 148-151, 154-156, 159-161, 163-165, 168-170, 177, 179, and 183-194 have been rejected under 35 USC 103(a) as being unpatentable over Huse in view of Miyota. This rejection is respectfully traversed.

The present rejection must fall as Miyota is not available as a reference against this application. Miyota was published on February 12, 1998, which is long after the U.S. filing date of the present application, which is November 4, 1997. It is not even necessary to rely on the earlier priority date of applicant's Israeli application of November 7, 1996. Accordingly, as one of the publications relied upon by the examiner to make this rejection is not available as prior art to this application, this art rejection must fall.

Reconsideration and withdrawal thereof is therefore respectfully urged.

It is submitted that all of the claims now present in the case clearly define over the references of record and fully comply with 35 U.S.C. §112. Reconsideration and allowance are therefore earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By _____
Roger L. Browdy
Registration No. 25,618

RLB:jab
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
G:\BN\R\ramq\gershoni5\Pto\AmendmentI.doc